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Research Article

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Very Low Density Lipoprotein Triglyceride Transport in Type IV Hyperlipoproteinemia and the Effects of Carbohydrate-Rich Diets

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ABSTRACT Transport of plasma-free fatty acids (FFA) and of fatty acids in triglycerides of plasma very low density lipoproteins (VLDL-TGFA) was studied in two normal subjects, five patients with type IV hyperlipoproteinemia, and two patients with type I hyperlipoproteinemia. After intravenous pulse-labeling with albumin-bound 1-palmitate-¹⁴C, specific radioactivity of plasma FFA and VLDL-TGFA were determined at intervals up to 24 hr. The results were analyzed using several different multicompartmental models each compatible with the experimental data. Fractional transport of VLDL-TGFA was distinctly lower (no overlap) in the type IV patients than in the control subjects, both on a usual balanced diet (40% of calories from carbohydrate) and on a high-carbohydrate diet (80% of calories). However, net or total transport of VLDL-TGFA in the type IV patients was not clearly distinguishable from that in the control subjects, there being

considerable overlap on either diet. The results suggest that in this group of type IV patients the underlying defect leading to the increased pool size of VLDL-TGFA is not overproduction but a relative defect in mechanisms for removal of VLDL-TGFA. Since some of these type IV patients had only a moderate degree of hypertriglyceridemia at the time they were studied, and since it is not established that patients with the type IV phenotype constitute a biochemically homogeneous population, the present results should not be generalized.

Four studies were done (in two control subjects and two type IV patients) in which the kinetic parameters in the same individual were determined on the balanced diet and on the high-carbohydrate diet. All subjects showed an increase in VLDL-TGFA pool size. Using two of the models for analysis, all showed an increase in net transport of VLDL-TGFA; using the third model, three of the four studies showed an increase in VLDL-TGFA transport. The results are compatible with the interpretation that the carbohydrate-induced increase in VLDL-TGFA, both in controls and type IV patients, is at least in part due to an increased rate of production of VLDL-TGFA. The magnitude of the increase was approximately the same in controls and patients. Thus, metabolic adjustment to a high-carbohydrate regimen in these type IV patients may not be basically different from that in normal controls; the higher levels of VLDL-TGFA reached may simply be another reflection of a defective removal mechanism. An alternative interpretation, compatible with the data, would involve both a carbohydrate-induced increase in fractional rate of release of VLDL-TGFA from liver to plasma and a decrease in fractional removal of VLDL-TGFA from plasma without increase in net

A preliminary report of these results was presented at the Deuel Conference, Carmel, Calif., Feb. 21-24, 1968 and published in *The Turnover of Lipids and Lipoproteins*. G. Cowgill, D. L. Estrich, and P. D. Wood, editors. Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 95-102. The report was presented before the Western Society for Clinical Research, Carmel, Calif., 29 January 1970 (*Clin. Res.* 18: 187).

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production rate. The simpler hypothesis of a single primary effect on net VLDL-TGFA production from FFA seems more likely.

INTRODUCTION

Hyperlipoproteinemia of the type IV variety is a relatively common disorder, believed to predispose to development of premature coronary artery disease (1). While the degree of hyperlipoproteinemia can be greatly modified by appropriate diets, there is strong evidence that the patients with this disorder have a genetically determined error in metabolism. In designing experimental approaches to elucidation of the metabolic error it would obviously be important to determine whether the elevated very low density lipoprotein (VLDL)¹ levels are the result of an increased rate of production, a decreased rate of removal, or both. The present studies were undertaken to compare rates of VLDL triglyceride turnover in normal controls and in patients with type IV hyperlipoproteinemia, both on diets of usual composition and on diets rich in carbohydrate.

Triglyceride transport has been studied by a number of investigators using a variety of experimental techniques and a variety of approaches in interpretation of kinetic data (2-9). In most of these studies data were collected for 5 hr, or less, on the supposition that all or most of the rate constants involved in transport of triglycerides are relatively large and would be apparent within that time interval (2-4, 8, 9). In such short-term studies the contribution of a slow process, not necessarily evident from inspection and analysis of the tracer curve yet contributing significantly to mass transport, might be overlooked. Calculation of fractional catabolic rate from the initial slope of the declining triglyceride specific activity curve, where it may appear to be first-order, similarly entails the assumption that the fractional rate of glyceride removal is appreciably less than the fractional turnover rates of metabolites in the precursor systems and that the disappearance curve will remain first-order (2, 3, 5-7, 10). In the present studies, specific radioactivity of plasma FFA and VLDL triglycerides was followed for periods up to 24 hr after intravenous injection of labeled FFA. The early descending limb of the curve for VLDL specific activity did not remain first-order but showed instead a definite flattening at later times, as noted previously by Havel (11).

The data were analyzed using three different multi-compartmental models. These were designed on the basis of current concepts of triglyceride transport and modified as necessary to maximize concordance with the

present experimental results. The three models developed all gave results in excellent concordance with the present data, but no single model was totally satisfactory when related to the data available in the literature regarding glyceride transport (see accompanying paper [12]). The parametric results obtained using each of the three models are presented, and the differences are discussed.

METHODS

1-Palmitic acid-¹⁴C (27.5 mCi/mmmole) was obtained from the New England Nuclear Corp. (Boston, Mass.). To establish purity, gas-liquid chromatography (17% polyethylene glycol succinate on Chromosorb W) was performed on the methyl ester of the labeled fatty acid. More than 98.5% of the recovered counts were found in the methyl palmitate fraction. On thin-layer chromatography (Silica Gel G; petroleum ether:diethyl ether:glacial acetic acid, 90:10:1), more than 99% of the applied counts were recovered in the free fatty acid fraction. The labeled palmitate was complexed to albumin (5% solution) (13), made to a concentration of 10 μ Ci/ml with saline, and sterilized by Millipore filtration.

13 studies were performed on nine individuals (Table I). Two normal individuals were studied both on a balanced diet and on a high-carbohydrate diet (studies 1a,b and 2a,b). Two patients with elevated prebeta lipoprotein triglycerides (type IV hyperlipoproteinemia) were also evaluated both on a balanced diet and on a high-carbohydrate diet (studies 6a,b and 12a,b); three additional type IV patients were studied on only one occasion, either on balanced diet (Table I, study 10a) or on high-carbohydrate diet (studies 3b and 4b). Finally, two patients with type I hyperlipoproteinemia were studied, one while on a balanced diet (study 5) and the other while on a high-carbohydrate diet (study 11). Caloric levels were adjusted to maintain constant body weight. The balanced diet provided 20% of calories from protein, 40% from fat, and 40% from carbohydrate; the high-carbohydrate diet provided approximately 20% of calories from protein, 80% from carbohydrate, and less than 1% from fat. The studies were performed under close dietary control while each subject was an inpatient at the Clinical Center of the National Institutes of Health. Weights were maintained within 1 or 2 kg of the mean basal weight during the month preceding the study. The diets were fed for 1 wk to 10 days before the kinetic studies, and fasting serum triglyceride values were determined serially. The kinetic studies were begun only after stable plasma triglyceride levels had been achieved. Conventional glucose-tolerance tests were performed on each of the subjects after adequate preparation (at least 300 g carbohydrate daily for 4 days prior to testing), and the results were interpreted using the criteria of Fajans and Conn (14).

The classification of the patients (Table I) was based on the criteria of Fredrickson, Levy, and Lees (1). The triglyceride levels shown in Table I were those at the time of the tracer studies and were only slightly elevated in subjects 6 and 10. Subject 6, on balanced diet, had total plasma triglyceride levels ranging from 144 to 252 mg/dl with a mean of 186; on high-carbohydrate diet values ranged from 270 to 396 mg/dl with a mean of 343. In subject 10 values on balanced diet ranged from 163 to 411 mg/dl with a mean of 307; on high-carbohydrate diet values ranged from 436 to 670 mg/dl with a mean of 522. Subject 3 when first studied in the Clinical Center showed a typical

¹Abbreviations used are: FFA, free fatty acid(s); TGFA, triglyceride fatty acid(s); VLDL, very low-density lipoprotein(s) ($d < 1.006$); LDL, low density lipoprotein(s) ($d 1.006-1.063$).

TABLE I
Clinical Characteristics of Subjects

Study No.	Age	Sex	Weight	Diet	Lipoprotein pattern	Total plasma triglyceride concentration	Glucose* tolerance test
			kg			mg/dl	
1 a	19	M	68.5	Balanced	Normal	78	—
1 b	19	M	68.5	High-carbohydrate	Normal	100	Normal
2 a	18	F	54.3	Balanced	Normal	31	—
2 b	18	F	56.1	High-carbohydrate	Normal	110	Normal
3 b	37	M	79.2	High-carbohydrate	"Type IV"†	110	Normal
4 b	43	M	85.0	High-carbohydrate	Type IV	930	Diabetic
5 a	25	M	58.9	Balanced	Type I	1440	Normal
6 a	37	M	80.6	Balanced	Type IV	154	—
6 b	37	M	81.9	High-carbohydrate	Type IV	360	Diabetic
10 a	56	M	70.4	Balanced	Type IV	163	Diabetic
11 b	20	M	62.5	High-carbohydrate	Type I	168	Normal
12 a	39	M	59.5	Balanced	Type IV	395	—
12 b	39	M	59.8	High-carbohydrate	Type IV	480	Diabetic

* A 100 g oral glucose load was given and the response evaluated using criteria of Fajans and Conn (14).

† This patient was classified as type IV previous to the present study, at a time when the patient was grossly obese and showed a diabetic glucose tolerance test. In the interim he lost 50 kg, restored his glucose tolerance to normal, and reduced his plasma triglycerides to normal.

type IV lipoprotein pattern and an abnormal glucose tolerance test, but at the time of study he had lost 50 kg with the return of glucose tolerance to normal and normalization of his VLDL level. After the present studies were completed he gained back 15 kg, his glucose tolerance became again abnormal and his VLDL level rose again.

The subjects fasted overnight (14 hr) before the injection of labeled FFA. Bed rest was maintained on the morning of the study. 2 hr after the palmitate injection, a fat-free breakfast was allowed. The two remaining meals during the day were also fat free. With these fat-free feedings plasma triglyceride levels were stable over the course of the 24 hr except in study 2b where, after 7 hr, the level fell by about 33%. In preliminary studies done during a total fast for 24 hr, TGFA levels fell progressively. Large indwelling plastic catheters were placed in the antecubital veins of both arms, and 7–10 μ Ci of 1-palmitate- 14 C-albumin complex was injected rapidly into one of them. Blood samples were obtained from the opposite arm at 2-min intervals for the first 20 min and then at less frequent intervals to 24 hr. All blood was collected in disodium EDTA (1 mg/ml whole blood) and placed immediately in ice. Plasma was separated from cells in a refrigerated International PR-2 Centrifuge (International Equipment Co., Needham Heights, Mass.) at 2000 rpm for 25 min. 1 ml aliquots of plasma were extracted immediately for free fatty acid determination, and the remaining plasma was stored briefly at 4°C before the isolation of the very low density proteins. Exchange of VLDL-TGFA label with that in other lipoproteins and net transfer of VLDL-TGFA were both shown to be negligible

at the temperature (4°C) and over the time interval involved in isolation. However, significant net transfer of VLDL-TGFA to higher density fractions occurred when samples were incubated for 4–6 hr at 37°C.

Previous clinical studies showed that plasma obtained during the first 20 min after injection of labeled palmitate contained radioactivity only in the free fatty acid fraction (8). This was confirmed by thin-layer chromatography of total lipid extracts in a few of the present studies; with the exception of a small fraction of label in a more polar contaminant (see below), all the activity was in FFA. 1 ml aliquots of plasma taken during the initial 20 min were extracted by the Dole procedure (15), and the radioactivity in the heptane phase was determined. Plasma obtained after 20 min was extracted similarly, but phospholipids were then removed from a chloroform solution of the extracted lipids by adsorption onto silicic acid (16). Triglycerides and cholesterol esters were separated from free fatty acids by the method of Borgström (17). The isolated free fatty acids were made to volume, and separate aliquots were assayed for radioactivity and titratable FFA. Titrations were performed by both automated² and manual methods (15).

Thin-layer chromatography of the FFA fraction in two studies (12a and b) revealed the presence of a labeled material more polar than fatty acids. This material had a smaller R_f value than that of palmitic acid in two different solvent systems (petroleum ether:diethyl ether:glacial acetic acid [90:10:1] and chloroform:methanol:water

² Greenough, W. B., III. Unpublished results.

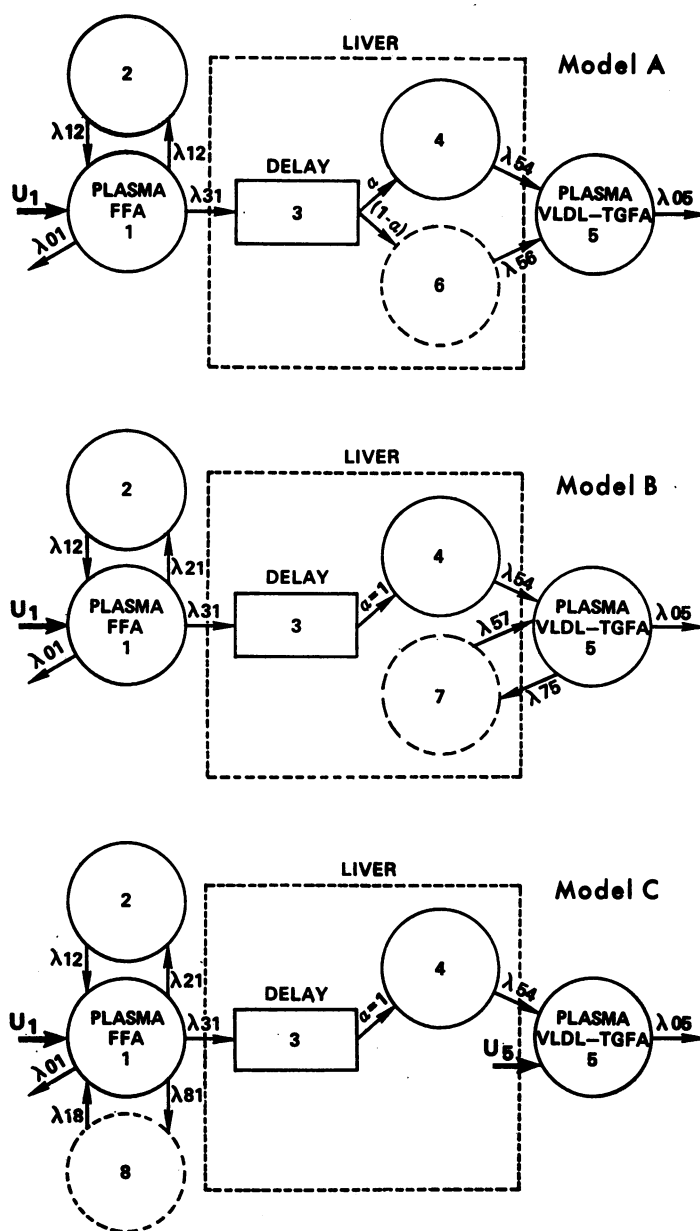


FIGURE 1 Three multicompartamental models utilized in analysis of kinetic data. The symbols used and their definitions are listed:

- M_j —Steady-state mass of compartment j in μEq .
- U_j —Steady-state transport of mass into compartment j from outside of system in $\mu\text{Eq min}^{-1}$.
- λ_{ij} —Fractional rate of transport of activity or mass to compartment i from compartment j in min^{-1} .
- λ_{0j} —Fractional rate of irreversible transport of activity or mass out of system from compartment j in min^{-1} .
- $\lambda_{j\bar{j}}$ —Fractional rate of irreversible and reversible transport of activity or mass out of compartment j in min^{-1} .
- ρ_{ij} —Steady-state transport of mass to compartment i from compartment j in $\mu\text{Eq min}^{-1}$.
- ρ_{0j} —Irreversible steady-state transport of mass out of system from compartment j in $\mu\text{Eq min}^{-1}$.

[195:75:12]). During the first 90 min the radioactivity in this more polar fraction was always less than 15% of the total. In later samples, however, when the FFA radioactivity had reached very low levels, larger fractions of radioactivity were found in the polar fraction, and the FFA radioactivity data were not considered reliable beyond 90 min. No FFA data beyond this time were used in kinetic analysis.

Very low density lipoproteins were isolated in a Spinco Model L ultracentrifuge at 4°C using a 40.3 rotor. The plasma was layered under 0.85% NaCl (density 1.006) and spun at 40,000 rpm for 16 hr. Very low density lipoproteins were collected from the top of the tube with a slicer and resuspended prior to extraction. Low density lipoproteins (LDL) were prepared from the infranant fraction by adjusting the density to 1.063 and centrifuging for 20 hr at 40,000 rpm. Chylomicrons were absent from fasting plasma both in the normal subjects and in the patients with type IV hyperlipoproteinemia. This was checked by repeated paper electrophoretic studies and by centrifuging at 3000 rpm for 30 min and inspecting for increased turbidity at the surface. In the two patients with type I hyperlipoproteinemia (Table I, studies 5 and 11) chylomicrons were first removed by layering plasma under 0.85% saline and centrifuging for 25 min at 20,000 rpm. After the chylomicrons had been removed, the very low density lipoproteins were isolated as described above. These were resuspended, layered under 0.85% saline and again centrifuged (20,000 rpm for 25 min) to insure complete removal of chylomicrons. The chylomicrons isolated in the first centrifugation were extracted, and their triglycerides were isolated by the same methods used for very low density lipoprotein triglycerides.

The very low density lipoproteins were extracted with the mixture described by Dole (15), 5 vol/vol of lipoprotein solution. The lipid extract was dried under nitrogen, redissolved in CHCl_3 , and a silicic acid adsorption of phospholipids was carried out (16). FFA were removed by partitioning against alkaline ethanol. The final extract in all samples was examined by thin-layer chromatography in two studies. Contamination of the glyceride fraction with radioactivity in other lipids never exceeded 4%; radioactivity attributable to phospholipid averaged 1.5%, and that due to cholesterol esters averaged 1%. Triglyceride mass was determined by the automated fluorometric technique of Kessler and Lederer (18) and was expressed as microequivalents of triglyceride fatty acid (TGFA) per milliliter, using pure tripalmitin as a reference standard.

All radioactivity was assayed in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), using 0.5% diphenyloxazole in toluene as the scintillation solvent. Quenching corrections were determined using

an external standard, but the quenching was minimal and could usually be neglected. Counts were corrected for efficiency and expressed as disintegrations per minute. Both the FFA and TGFA specific activities in each study were normalized to a constant injected dose of radioactive FFA and expressed as fraction of injected dose per microequivalent of FFA or microequivalent of very low density lipoprotein TGFA.

The final data were analyzed using a number of different models compatible with current concepts of the metabolism of FFA and VLDL triglyceride (19, 20). Previous experience of other investigators was used as a guide in selecting appropriate models (8, 21). The criteria applied in selecting and evaluating models and in comparing alternative models are present in detail in the accompanying paper by Shames et al. (12). Three models equally compatible with tracer and tracee data were developed (Fig. 1). The parameters evaluated and the abbreviations used are listed in the legend to Fig. 1. The pool sizes for plasma FFA and VLDL-TGFA (M_1 and M_6 , respectively) were calculated from the measured plasma concentrations and the estimated plasma volume (41.3 ml/kg of body weight). These three models differ only in the element introduced to account for the radioactivity persisting in VLDL-TGFA for prolonged periods of time, i.e., to account for the late portion of the VLDL-TGFA curve. In model A this is effected by a slow pathway for conversion of FFA to VLDL-TGA (compartment 6) in addition to the relatively rapid pathway common to the other models (compartment 4). Model C has only one conversion pathway (compartment 4) but provides for a sustained flow of FFA radioactivity entering it as a result of the slow return of FFA from an additional FFA exchange pool (compartment 8). To satisfy steady-state relationships in this model, an additional source of VLDL-TGFA production from unlabeled precursor had to be incorporated (U_6). In model B the plasma VLDL-TGFA recycles with an extravascular pool (compartment 7, not necessarily within the liver as shown) and thus sustains the plasma VLDL-TGFA at later times.

Some of the parameters (see legend to Fig. 1 for definitions of symbols) are equivalent in each of the models (e.g., λ_{06} and ρ_{64}); ρ_{06} in model A, ρ_{67} in model B, and U_6 in model C are also equivalent. Others are obviously different depending on the model used, but as demonstrated in the accompanying manuscript (12) analogous parameters are affected similarly by perturbations in the system. The tabulated data presented in this paper are those calculated using model A. Direct comparisons of results using all three models are presented for each of the important transport rates in Figs. 3 and 5. The *relative* values of other key

ρ_{j1} —Irreversible and reversible steady-state transport of mass out of compartment j in $\mu\text{Eq min}^{-1}$.

Symbols relating to FFA and TGFA transport:

M_1 —Plasma FFA mass in μEq .

M_6 —Plasma VLDL-TGFA mass in μEq .

λ_{06} —Fractional rate of turnover of plasma VLDL-TGFA in min^{-1} .

λ_{31} —Fractional rate of incorporation of plasma FFA into plasma VLDL-TGFA in $\mu\text{Eq min}^{-1}$.

ρ_{06} —Steady-state rate of production of VLDL-TGFA in $\mu\text{Eq min}^{-1}$.

ρ_{31} —Steady-state rate of incorporation of plasma FFA into plasma VLDL-TGFA in $\mu\text{Eq min}^{-1}$.

U_1 —Steady-state rate of delivery of FFA (nonrecycling) into plasma in $\mu\text{Eq min}^{-1}$.

U_6 —Steady-state rate of delivery of unlabeled VLDL-TGFA into plasma in $\mu\text{Eq min}^{-1}$.

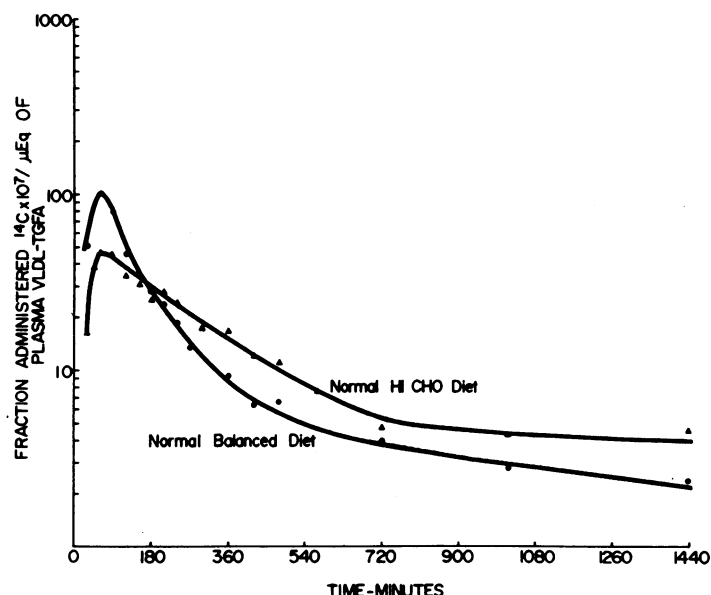


FIGURE 2 Specific radioactivity of VLDL-TGFA as a function of time after intravenous administration of 1-palmitate- ^{14}C . Results in normal control subject 2 are shown both on a balanced diet and on a high-carbohydrate diet.

parameters are affected similarly by high-carbohydrate diet regardless of which of the three models is considered. Comparison between control subjects and type IV patients on a given diet also yield similar qualitative differences irrespective of the model used. Finally, it is essential to note again that the three models under discussion gave equally satisfactory fits with the experimental data and that more complex models combining two or all three of the "slow pathway" components (compartments 6, 7, and 8) would also give satisfactory fits.

The SAAM 24 digital computer program (22) was used to obtain least squares fits of data and confidence limits, expressed in tabulated data as per cent standard deviation. Constraints used for the model solutions were those discussed in the accompanying paper (12).

RESULTS

Normal subjects. The plasma FFA specific activity curves in two normal individuals were not significantly affected by diet. However, the proportion of total FFA transport directed toward TGFA synthesis (λ_{s1} in relation to $\lambda_{s1} + \lambda_{o1}$) was increased as discussed below. The FFA data were analyzed only to 90 min; after this the low total radioactivities and the presence of a polar radioactive contaminant made accuracy questionable.

The TGFA specific activity curves were manifestly altered in subject 2 by the high-carbohydrate diet (Fig. 2). On a balanced diet the curve rose promptly after the usual 30 min delay, reached a peak between 1 and 2 hr, and then showed a rapid decline. Of special note is the break in the curve at about 8 hr, followed by a distinctly slower decay beyond 8 hr. On the high-carbohydrate

diet the initial peak was lower and the initial decay was slower, especially between 2 and 8 hr. The distribution of the area under the curve on the high-carbohydrate diet was clearly shifted to the right. Qualitatively similar curves were obtained in subject 1, but the effect of carbohydrate feeding on the decay between 2 and 8 hr was minimal.

Values of pertinent fractional rate constants, pool sizes, and transport rates derived from application of model A to these data are presented in Table II. The fractional rate of plasma FFA entering the triglyceride synthetic pathway (λ_{s1}) was increased with high-carbohydrate feeding in both studies 1 and 2 by 57 and 44%, respectively. There was a decrease in the fractional rate of irreversible loss of plasma FFA to pathways other than VLDL-TGFA synthesis (λ_{o1}). However, the total irreversible fractional transport of FFA ($\lambda_{s1} + \lambda_{o1}$) changed only slightly. A 50% decrease in the fractional rate of VLDL triglyceride catabolism (λ_{o6}) occurred in study 2, but the change in subject 1 was insignificant.

The net transport (ρ) of FFA and VLDL-TGFA also appeared to be altered in the normal subjects under conditions of high-carbohydrate feeding. Model A is so constructed that the net transport of plasma VLDL-TGFA, ρ_{o5} , equals that of FFA into the TGFA synthetic pathway, ρ_{s1} . An increased net transport of plasma FFA into the VLDL-TGFA synthetic pathway occurred; thus, ρ_{s1} (and ρ_{o5}) increased from 80 to 111 μEq of FFA min^{-1} in study 1, and from 32 to 51 μEq of

TABLE II
Results in Two Normal Subjects on Balanced Diet (a) and on High-Carbohydrate Diet (b)*

Parameters	Study 1a	Study 1b	Percentage change	Study 2a	Study 2b	Percentage change
	min^{-1}			min^{-1}		
λ_{01}	0.22 ($\pm 6\%$)†	0.15 ($\pm 6\%$)	-35	0.14 ($\pm 10\%$)	0.11 ($\pm 17\%$)	-21
λ_{31}	0.039 ($\pm 33\%$)	0.061 ($\pm 31\%$)	+57	0.021 ($\pm 15\%$)	0.030 ($\pm 15\%$)	+44
λ_{05}	0.018 ($\pm 33\%$)	0.016 ($\pm 31\%$)	-8	0.017 ($\pm 15\%$)	0.0073 ($\pm 15\%$)	-57
	$\mu\text{Eq FFA or TGFA}$			$\mu\text{Eq FFA or TGFA}$		
M_5	4480	6780	+51	1880	7040	+270
M_1	2040	1800	-15	1540	1720	+12
M_4	2740 ($\pm 55\%$)	2790 ($\pm 54\%$)		<400§	<400§	
M_6	70,000 ($\pm 53\%$)	355,000 ($\pm 36\%$)	+410	206,000 ($\pm 26\%$)	258,000 ($\pm 28\%$)	+25
	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$			$\mu\text{Eq FFA or TGFA}$		
U_1	534 ($\pm 4\%$)	374 ($\pm 4\%$)	-30	243 ($\pm 6\%$)	237 ($\pm 10\%$)	-2
ρ_{01}	454 ($\pm 6\%$)	264 ($\pm 6\%$)	-42	211 ($\pm 10\%$)	160 ($\pm 17\%$)	-24
$\rho_{05} \equiv \rho_{31}$	80 ($\pm 33\%$)	111 ($\pm 31\%$)	+40	32 ($\pm 15\%$)	51 ($\pm 15\%$)	+61
ρ_{54}	41 ($\pm 31\%$)	42 ($\pm 32\%$)	+2	9 ($\pm 15\%$)	13 ($\pm 12\%$)	+46
ρ_{56}	38 ($\pm 37\%$)	69 ($\pm 33\%$)	+80	23 ($\pm 16\%$)	38 ($\pm 18\%$)	+69

* a and b represent studies on balanced diet and on high-carbohydrate diet, respectively; values calculated based on use of model A.

† Values in parentheses indicate estimated relative standard deviation of each parameter.

§ Compartment 4 was too small to be determined exactly.

FFA min^{-1} in study 2 (Table II). It is important to note that the larger increment in plasma VLDL-TGFA

pool size (M_6) resulting from high-carbohydrate feeding occurred in study 2, where there was a significant decrease in λ_{05} . The increment in VLDL-TGFA transport (ρ_{05}) associated with carbohydrate feeding in study

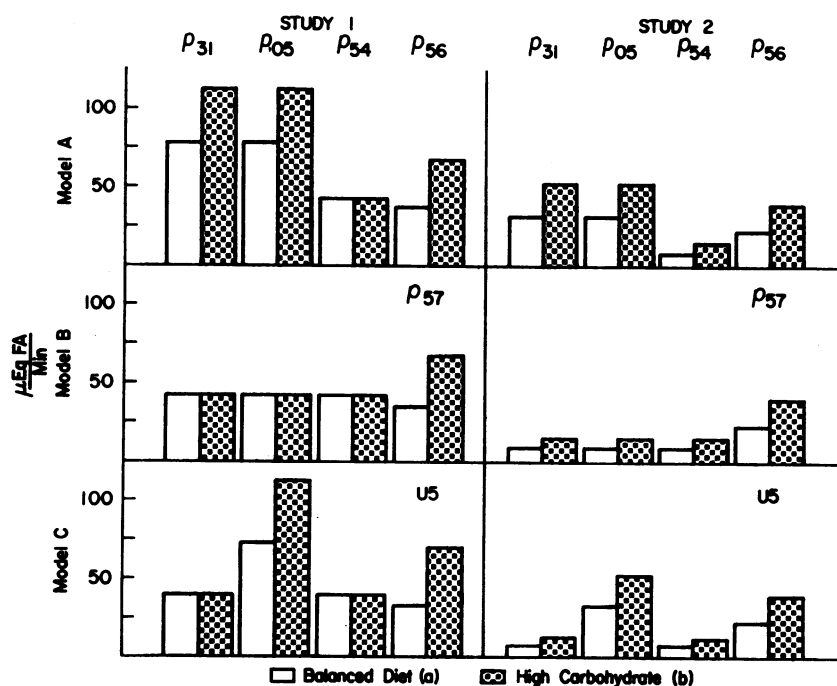


FIGURE 3 Normals: dietary alterations of transport. Calculated transport functions (ρ) for two normal subjects studied on a balanced diet (\square) and on a high-carbohydrate diet (checked bars). Computer analysis was carried out using all three of the models shown in Fig. 1. See text for discussion.

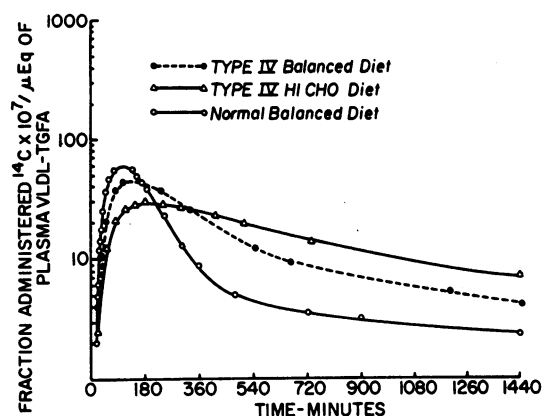


FIGURE 4 Specific radioactivity curves for VLDL-TGFA in type IV, subject 6 on balanced diet (●) and on high-carbohydrate diet (△). The curve for normal subject 1 on balanced diet (○) is shown for comparison.

2 was no greater than that in study 1 where much less expansion of VLDL-TGFA pool size (M_5) occurred. Proportionately more of the increased FFA incorporation into VLDL-TGFA secondary to high-carbohydrate feeding occurred via the slower conversion pathway (ρ_{05}) than by the more rapid pathway (ρ_{54}) (Table II). The size of the slowly turning over TG pool in the liver, M_5 , increased from 18 g in study 1a to 91 g in study 1b. The magnitude of this hypothetical precursory glyceride pool in the liver is extremely large and probably is inconsistent with experimental data on hepatic glyceride content. This is discussed more fully below.

The data obtained in the normal studies were also evaluated using models B and C (Fig. 1). Model B incorporates a recycling plasma-liver system for the VLDL-TGFA (pool 7). Model C incorporates a slowly turning over FFA distribution system, as well as a source of unlabeled VLDL-TGFA (U_5). The calculated transport rates (ρ , in microequivalents per minute) derived by application of models A, B, and C are shown in Fig. 3. The three models lead to substantially different *absolute* values for some of the measures of FFA-TGFA transport. Using models A or C, one calculates an increase in net transport of VLDL-TGFA (ρ_{05}) in both studies. According to model A, this is associated with a diversion of a larger fraction of FFA to the hepatic TGFA synthetic pathway (ρ_{31}); according to model C, it is associated with an increased input from an unlabeled source, U_5 . Using model B, one again finds an increased VLDL-TGFA transport in study 2 but not in study 1. In the latter, the exchange with the postulated hepatic pool increased (ρ_{07}), but ρ_{05} did not increase.

Patients with type IV hyperlipoproteinemia. The plasma VLDL triglyceride specific activity curves in both studies 6 and 12 demonstrated changes somewhat similar to those observed in normal subjects following high-carbohydrate feeding (Fig. 4). The normal plasma VLDL triglyceride specific activity curve on the balanced diet (study 1a) is presented for comparison.

The rate constants, transports, and pool sizes for the two patients with type IV hyperlipoproteinemia on a balanced diet and on a high-carbohydrate diet (calcu-

TABLE III
Results in Two Patients with Type IV Hyperlipoproteinemia on Balanced Diet (a) and on High-Carbohydrate Diet (b)*

Parameters	Study 6a	Study 6b	Percentage change	Study 12a	Study 12b	Percentage change
	<i>min⁻¹</i>			<i>min⁻¹</i>		
λ_{01}	0.274 ($\pm 6\%$)†	0.090 ($\pm 28\%$)	-67	0.075 ($\pm 42\%$)	0.100 ($\pm 42\%$)	+33
λ_{31}	0.062 ($\pm 26\%$)	0.094 ($\pm 27\%$)	+52	0.062 ($\pm 48\%$)	0.085 ($\pm 48\%$)	+38
λ_{05}	0.0075 ($\pm 26\%$)	0.0033 ($\pm 27\%$)	-57	0.0035 ($\pm 48\%$)	0.0035 ($\pm 48\%$)	0
	<i>μEq FFA or TGFA</i>			<i>μEq FFA or TGFA</i>		
M_5	10,100	37,700	+270	21,900	32,500	+48
M_1	1230	1320	+7	1230	1330	+8
M_4	2380 ($\pm 21\%$)	2870 ($\pm 18\%$)	+21	1270 ($\pm 26\%$)	1860 ($\pm 24\%$)	+46
M_6	46,600 ($\pm 36\%$)	219,000 ($\pm 35\%$)	+370	104,000 ($\pm 52\%$)	155,000 ($\pm 52\%$)	+50
	<i>μEq min⁻¹ FFA or TGFA</i>			<i>μEq min⁻¹ FFA or TGFA</i>		
U_1	414 ($\pm 3\%$)	242 ($\pm 3\%$)	-42	167 ($\pm 5\%$)	245 ($\pm 4\%$)	+48
ρ_{01}	338 ($\pm 6\%$)	119 ($\pm 28\%$)	-65	92 ($\pm 42\%$)	132 ($\pm 42\%$)	+43
$\rho_{05} = \rho_{31}$	76 ($\pm 26\%$)	123 ($\pm 27\%$)	+62	76 ($\pm 48\%$)	113 ($\pm 48\%$)	+50
ρ_{54}	32 ($\pm 14\%$)	39 ($\pm 12\%$)	+20	22 ($\pm 11\%$)	32 ($\pm 9\%$)	+46
ρ_{56}	44 ($\pm 40\%$)	85 ($\pm 36\%$)	+92	54 ($\pm 66\%$)	81 ($\pm 66\%$)	+50

* a and b represent studies on balanced and on high-carbohydrate diets, respectively, using model A.

† Values in parentheses indicate estimated relative standard deviation of each parameter.

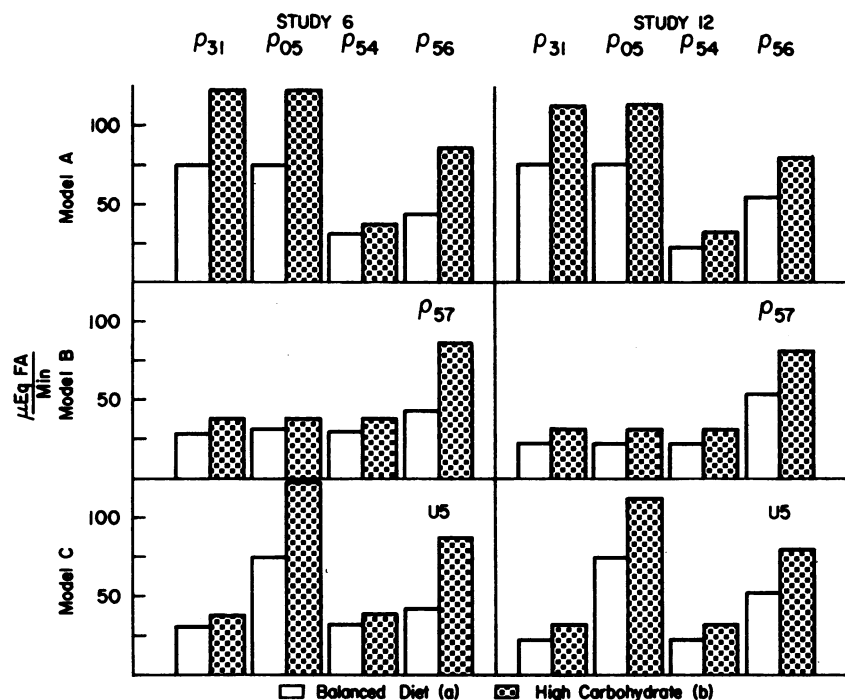


FIGURE 5 Type IV hyperlipoproteinemia: dietary alterations of transport. Calculated transport functions (ρ) for two subjects with type IV hyperlipoproteinemia on balanced diet (\square) and on high-carbohydrate diet (checked bars). Calculated values are shown as derived from the use of all three models shown in Fig. 1. See text for discussion.

lated using model A) are shown in Table III. The fractional rate of plasma FFA entry into the VLDL triglyceride conversion system (λ_{31}) increased on the

high-carbohydrate diet as it did in the normal subjects. The net rate of incorporation of plasma FFA into VLDL-TGFA (ρ_{31}) also increased in response to carbo-

TABLE IV
Results in Three Additional Patients with Type IV Hyperlipoproteinemia: Unpaired Dietary Studies

Parameters	Study 3b*	Study 4b*	Study 10a*
	min^{-1}	min^{-1}	min^{-1}
λ_{01}	0.17 ($\pm 18\%$) \ddagger	0.14 ($\pm 85\%$)	0.12 ($\pm 27\%$)
λ_{31}	0.032 ($\pm 21\%$)	0.11 ($\pm 106\%$)	0.051 ($\pm 10\%$)
λ_{05}	0.0049 ($\pm 21\%$)	0.0015 ($\pm 106\%$)	0.0032 ($\pm 10\%$)
	$\mu\text{Eq FFA or TGFA}$	$\mu\text{Eq FFA or TGFA}$	$\mu\text{Eq FFA or TGFA}$
M_5	12,400	102,000	14,700
M_1	1930	1330	920
M_4	774 ($\pm 29\%$)	2450 ($\pm 21\%$)	894 ($\pm 30\%$)
M_6	633,000 ($\pm 38\%$)	475,000 ($\pm 108\%$)	325,000 ($\pm 45\%$)
	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$
U_1	378 ($\pm 14\%$)	342 ($\pm 4\%$)	160 ($\pm 19\%$)
ρ_{01}	317 ($\pm 18\%$)	191 ($\pm 85\%$)	114 ($\pm 27\%$)
$\rho_{05} = \rho_{31}$	61 ($\pm 21\%$)	151 ($\pm 106\%$)	47 ($\pm 10\%$)
ρ_{54}	14 ($\pm 17\%$)	40 ($\pm 6\%$)	15 ($\pm 20\%$)
ρ_{56}	47 ($\pm 25\%$)	111 ($\pm 143\%$)	33 ($\pm 16\%$)

* a represents studies on a balanced diet, b on a high-carbohydrate diet.

\ddagger Values in parentheses indicate estimated relative standard deviation of each parameter.

TABLE V
Comparison of Results in Normal Subjects with Those in Patients with Type IV Hyperlipoproteinemia

	λ_{s1}	λ_{os}	M_1	M_5	$\rho_{05} = \rho_{s1}$	ρ_{s4}	ρ_{s5}
	min^{-1}	min^{-1}	$\mu\text{Eq FFA}$	$\mu\text{Eq TGFA}$	$\mu\text{Eq min}^{-1} \text{ TGFA or FFA}$		
Studies on balanced diet							
Normal							
1a	0.039 ($\pm 33\%$)*	0.018 ($\pm 33\%$)	2040	4480	80 ($\pm 33\%$)	41 ($\pm 31\%$)	38 ($\pm 37\%$)
2a	0.021 ($\pm 15\%$)	0.017 ($\pm 15\%$)	1540	1880	32 ($\pm 15\%$)	9 ($\pm 15\%$)	23 ($\pm 16\%$)
Type IV hyperlipoproteinemia							
6a	0.062 ($\pm 26\%$)	0.0075 ($\pm 26\%$)	1230	10,100	76 ($\pm 26\%$)	32 ($\pm 14\%$)	44 ($\pm 40\%$)
12a	0.062 ($\pm 48\%$)	0.0035 ($\pm 48\%$)	1230	21,900	76 ($\pm 48\%$)	22 ($\pm 11\%$)	54 ($\pm 66\%$)
10a	0.051 ($\pm 10\%$)	0.0032 ($\pm 10\%$)	1920	14,700	47 ($\pm 10\%$)	15 ($\pm 20\%$)	33 ($\pm 16\%$)
Studies on high-carbohydrate diet							
Normal							
1b	0.061 ($\pm 31\%$)	0.016 ($\pm 31\%$)	1800	6780	111 ($\pm 31\%$)	42 ($\pm 31\%$)	69 ($\pm 33\%$)
2b	0.030 ($\pm 15\%$)	0.0073 ($\pm 15\%$)	1720	7040	51 ($\pm 15\%$)	13 ($\pm 12\%$)	38 ($\pm 18\%$)
Type IV hyperlipoproteinemia							
6b	0.094 ($\pm 27\%$)	0.0033 ($\pm 27\%$)	1320	37,700	123 ($\pm 27\%$)	39 ($\pm 12\%$)	85 ($\pm 36\%$)
12b	0.085 ($\pm 48\%$)	0.0035 ($\pm 48\%$)	1330	32,500	113 ($\pm 48\%$)	32 ($\pm 9\%$)	81 ($\pm 66\%$)
3b	0.032 ($\pm 21\%$)	0.0049 ($\pm 21\%$)	1930	12,400	61 ($\pm 21\%$)	14 ($\pm 17\%$)	47 ($\pm 25\%$)
4b	0.110 ($\pm 106\%$)	0.0015 ($\pm 106\%$)	1330	102,000	151 ($\pm 106\%$)	40 ($\pm 6\%$)	111 ($\pm 143\%$)

* Estimated relative standard deviation of each parameter given in parentheses.

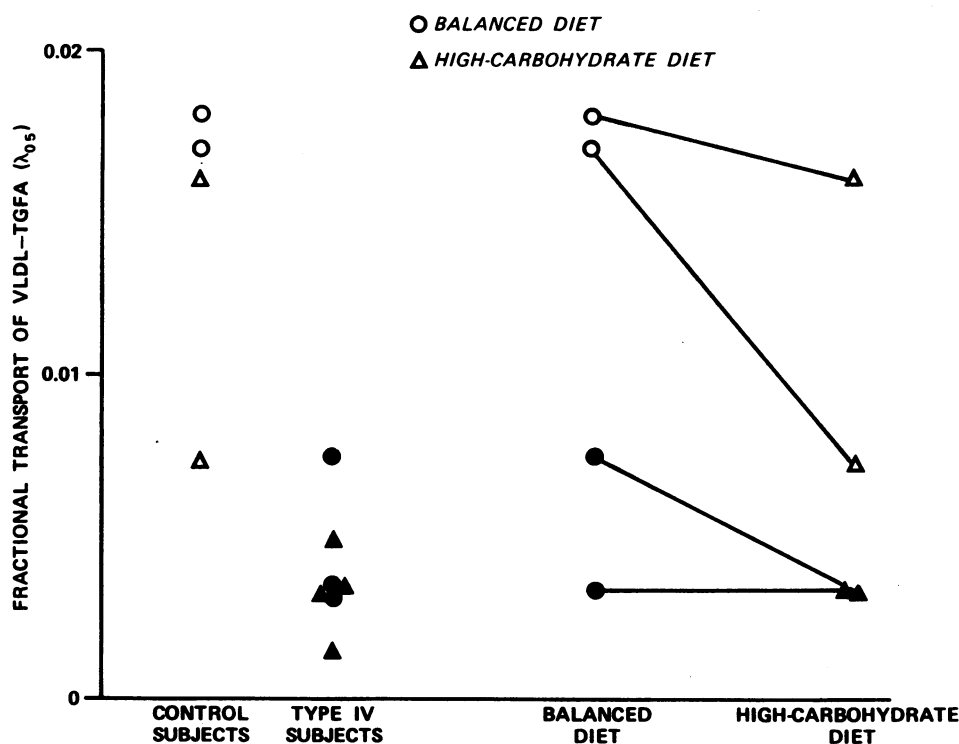


FIGURE 6 Comparison of fractional transport of VLDL-TGFA (λ_{05}) in control subjects (open symbols) and type IV subjects (closed symbols) on balanced diet (circles) and on high-carbohydrate diet (triangles). At the right are shown the shifts in the four paired studies induced by the high-carbohydrate diet.

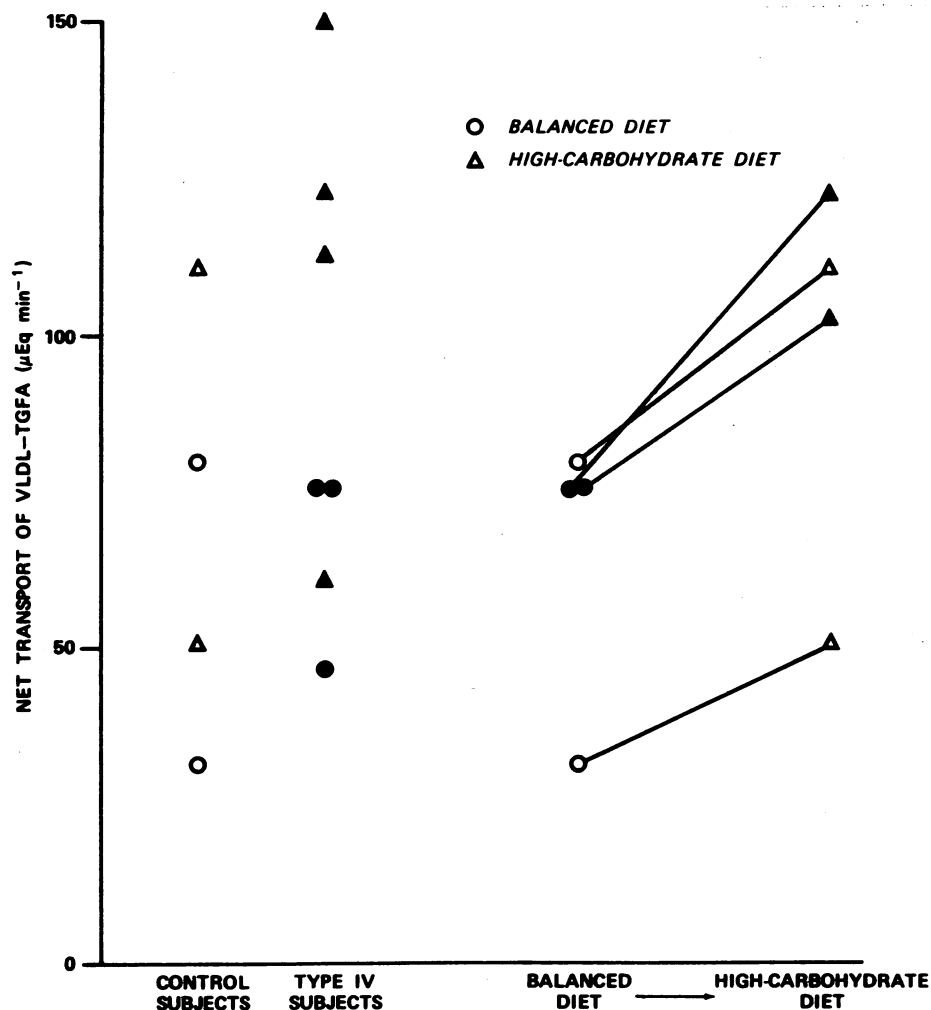


FIGURE 7 Comparison of *net transport* of VLDL-TGFA (ρ_{06}) in control subjects and type IV subjects (see legend to Fig. 6).

hydrate. As in the normal subjects, more of the increment went through the "slow" system in both studies (i.e., ρ_{06} was increased to a greater extent than ρ_{04}). Increases in the pool of VLDL-TGFA (M_5) occurred in both studies in response to dietary carbohydrate. The greater relative increment in pool size occurred in study 6 where a substantial decrease in λ_{05} was observed.

The two alternative models for VLDL-TGFA transport (i.e. models B and C) were also used in evaluating the data from studies 6 and 12. Again the absolute measures of FFA and TGFA transport were decidedly dependent on the model chosen (Fig. 5). For example, the calculated value for ρ_{05} (i.e., irreversible VLDL-TGFA transport) was much smaller using model B. Also, the magnitude of the increase in ρ_{05} caused by the high-carbohydrate diet was only about one-half as great using model B as it was using models A or C. How-

ever, the carbohydrate *effects* (i.e., relative changes) were qualitatively similar and independent of the model chosen.

Three additional studies were performed on type IV patients but without observation of dietary perturbations. These studies are summarized in Table IV.

A comparison of the FFA and VLDL-TGFA kinetics of normal subjects with those of type IV patients when on the same diets reveals a pattern of pertinent differences (Table V). On the balanced diet the type IV patients had fractional rates of plasma FFA incorporation into VLDL-TGFA (λ_{01}) greater than those in the normal subjects (Table V). However, the net transport of FFA into TGFA (ρ_{01}) and the coupled *absolute* transport of TGFA (ρ_{06}) were not consistently different from normal. On the high-carbohydrate diet, λ_{01} was also larger in the type IV patients, with the exception of

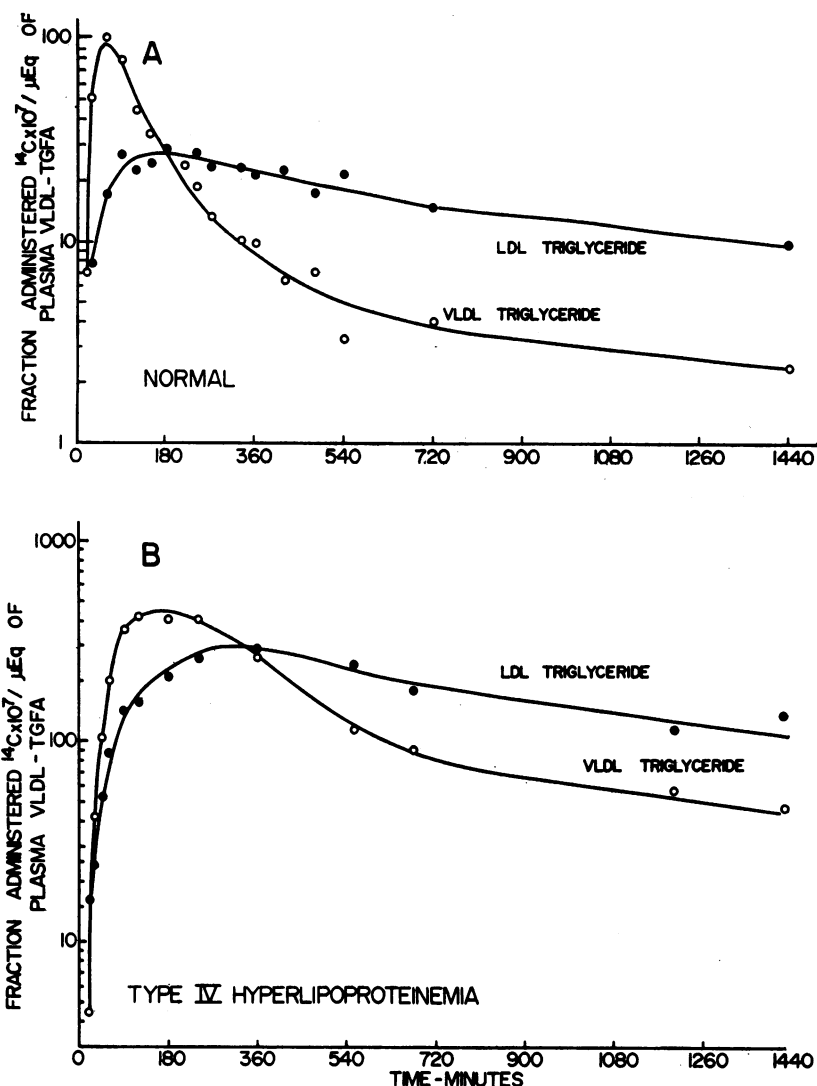


FIGURE 8 Representative examples of curves showing the precursor-product relationship between VLDL-TGFA and LDL-TGFA. A, results in a normal subject; B, results in a patient with type IV hyperlipoproteinemia.

subject 3. When this patient was first classified as type IV he was obese; he showed an abnormal glucose tolerance test and readily increased his VLDL-TGFA mass on a high-carbohydrate diet. At the time of the kinetic study, however, he had lost 50 kg, had normal glucose tolerance, and showed only a minimal increase in plasma triglyceride on a high-carbohydrate diet. Nevertheless, his *fractional* catabolic rate was below that seen in control subjects on the high-carbohydrate diet.

The other parameter significantly different between the two populations was the fractional catabolic rate for very low density lipoprotein triglyceride (i.e. λ_{os}). This parameter was consistently lower in the hyperlipemic patients on both the balanced diet and the high-carbo-

hydrate diet (Fig. 6). In the entire 13 studies the reciprocal correlation between λ_{os} and the size of the VLDL triglyceride pool size (M_s) was the single most striking relationship.

Despite the consistently lower values for λ_{os} in the type IV patients, *net* VLDL-TGFA transport was in the same range as that for normal subjects on either diet (Fig. 7). The mean value for transport of VLDL-TGFA (ρ_{os}) was slightly higher in the type IV individuals than in the control subjects, but it overlapped such that assignment of significance was precluded. Thus, in this limited series there was no evidence for increased over-all VLDL-TGFA transport in patients with endogenous hyperlipemia. The distribution of trans-

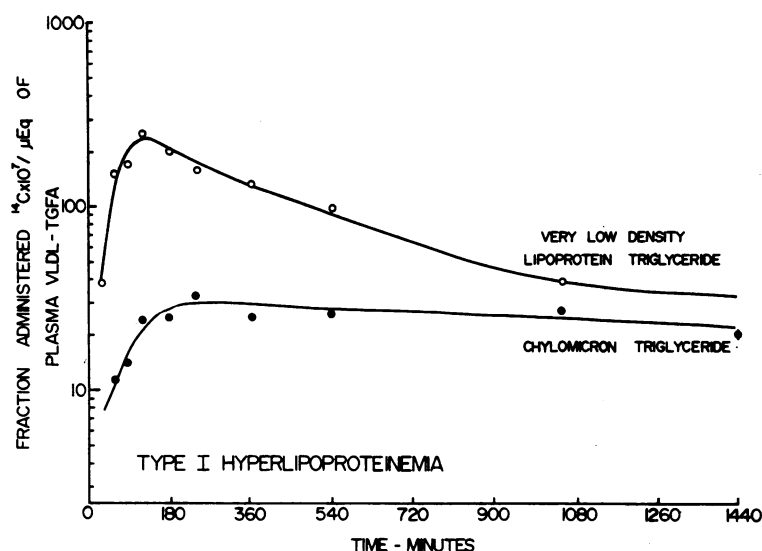


FIGURE 9 Relationship between the specific radioactivity curves of VLDL-TGFA and chylomicron-TGFA in a patient with type I hyperlipoproteinemia.

port between the slow pathway (ρ_{06}) and the fast pathway (ρ_{04}) was not significantly different between the two groups.

Triglyceride specific activity in LDL was also followed sequentially in each of the studies. A precursor-product relationship was consistently observed between VLDL and LDL-TGFA functions in both normal subjects and hyperlipemic subjects. Representative examples are shown in Fig. 8.

Type I hyperlipoproteinemia. The specific activity function for chylomicron triglycerides was significantly different from that for VLDL-TGFA in type I patients (Fig. 9). The peak specific activity reached was lower by an order of magnitude. The decay of chylomicron TGFA activity beyond the peak was much slower than that seen for VLDL-TGFA. No chylomicron triglyceride kinetic data were available in normal individuals to compare with this response, and no kinetic analysis was carried out.

The fractional transport of VLDL-TGFA (λ_{05} , Table VI) in the study on a balanced diet was low, in the same range as the values obtained in type IV patients. The VLDL-TGFA pool (M_5) was very large, and the net transport of VLDL-TGFA (ρ_{05}) was in the normal range. In the type I patient studied on a high-carbohydrate diet the VLDL-TGFA pool was no larger than it was in control subjects, and ρ_{05} was no higher than in controls.

DISCUSSION

Previous studies of VLDL-TGFA transport have usually been limited to data collected over the first 5 hr

after administration of labeled precursor (2-4, 7-9). Even when the VLDL-TGFA activity curve was followed for longer periods, the terminal slope was neglected in the analysis (5, 6, 10). In all the patients of this study, both normal and hyperlipemic, persisting VLDL-TGFA radioactivity was a prominent finding. In constructing a model, a continued slow input of VLDL-TGFA tracer into the plasma compartment was found necessary to simulate the experimental data in VLDL-TGFA. In each of the models this requirement was met in the following different ways (Fig. 1): in model A

TABLE VI
Results in Two Patients with Type I Hyperlipoproteinemia

Parameters	Study 5a*	Study 11b*
	min^{-1}	min^{-1}
λ_{01}	0.22 ($\pm 7\%$)†	0.15 ($\pm 8\%$)
λ_{21}	0.044 ($\pm 29\%$)	0.063 ($\pm 32\%$)
λ_{05}	0.0039 ($\pm 29\%$)	0.0068 ($\pm 32\%$)
	$\mu\text{Eq FFA or TGFA}$	$\mu\text{Eq FFA or TGFA}$
M_5	17,600	7080
M_1	1560	760
M_4	1390 ($\pm 31\%$)	1480 ($\pm 40\%$)
M_6	168,000 ($\pm 33\%$)	71,000 ($\pm 39\%$)
	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$	$\mu\text{Eq min}^{-1} \text{ FFA or TGFA}$
U_1	409 ($\pm 3\%$)	166 ($\pm 4\%$)
ρ_{01}	341 ($\pm 7\%$)	118 ($\pm 8\%$)
$\rho_{05} = \rho_{31}$	68 ($\pm 29\%$)	48 ($\pm 32\%$)
ρ_{04}	24 ($\pm 17\%$)	15 ($\pm 24\%$)
ρ_{06}	45 ($\pm 37\%$)	33 ($\pm 40\%$)

* a and b represent studies on balanced and on high-carbohydrate diets, respectively.

† Values in parentheses indicate estimated relative standard deviations of each parameter.

by introducing a slow FFA-TGFA conversion pathway ($3 \rightarrow 6 \rightarrow 5$); in model B by allowing for VLDL-TGFA recycling ($5 \rightleftharpoons 7$); and in model C by introducing a slow FFA recycling system ($1 \rightleftharpoons 8$). This postulated slow pathway in each case accounted for a large fraction of the total transport of VLDL-TGFA on a balanced diet in all individuals studied and demonstrated the greatest increment when a high-carbohydrate diet was given. Recycling of VLDL-TGFA through the plasma FFA pool could not in itself adequately account for this late portion of the VLDL curves (8, 12).

Another respect in which the models used in this analysis differ from those used in previous studies is with regard to the turnover characteristics of the precursor system. To best replicate the data in these studies it was necessary to incorporate a pool in the FFA-VLDL-TGFA conversion pathway with turnover characteristics similar to those of VLDL-TGFA itself (compartment 4). A model of this type yields higher fractional catabolic rate constants (λ_{06}) for VLDL-TGFA than calculated in studies in which all precursor turnover is considered to be significantly more rapid than that of the product.

There were substantial differences in some calculated rates of transport depending on the model used. For example, in subject 1 on a balanced diet, irreversible VLDL triglyceride transport (ρ_{06}) was calculated to be 80 $\mu\text{Eq}/\text{min}$ using model A or model C but only 43 $\mu\text{Eq}/\text{min}$ using model B. Although the absolute values for the model-dependent transport rates cannot be unambiguously decided without added information, perturbations due to diet produced analogous patterns of change *whichever model was used*. Moreover, the differences between normal subjects and hyperlipemic subjects were evident and analogous using any of the three models assuming the hypotheses set forth in the accompanying paper (12). One of these is the assumption that $\lambda_{5,4}$ is relatively invariant ($0.015 \text{ min}^{-1} \pm 20\%$) in all studies. However, equally satisfactory fitting of data could be obtained with different values for $\lambda_{5,4}$, and this resulted in significantly different values for $\lambda_{8,1}$ (and, indirectly, for $\lambda_{0,5}$ and $\rho_{8,1}$). Thus alternative interpretations are possible. We shall first discuss the results when $\lambda_{5,4}$ is held constant, and subsequently we will discuss how these may be modified with changes in $\lambda_{5,4}$.

The influence of the high-carbohydrate diet on free fatty acid transport in control subjects was quite consistent, and similar qualitative effects were observed in the patients with type IV hyperlipoproteinemia. An increased fractional rate of incorporation of plasma free fatty acid into the glyceride synthetic system ($\lambda_{8,1}$) was demonstrated in all four paired studies. In three of the four there was a concomitant reduction in FFA losses

through other pathways (λ_{01}) so that there were no consistent changes in irreversible FFA transport overall.

Using model A, the calculated fraction of irreversible FFA transport directed to VLDL-TGFA production averaged 23% on balanced diets (six studies) and 34% on high-carbohydrate diets (seven studies), but the difference was not statistically significant. In four cases on balanced diet the value was below 20%; two high values (29% and 45%) were obtained. Direct measurement of hepatic extraction of FFA by the liver in dogs has yielded values around 25–30% of total FFA transport (23–25), some of which is oxidized, stored, or resecreted as ketone bodies. These high values raise doubt as to the applicability of model A in those cases if the animal data are used as a guide. Using models B or C, the calculated values for VLDL-TGFA production as a fraction of irreversible FFA transport are about one-half those calculated using model A. Models combining features of all three would give intermediate values, as discussed below.

The net transport of VLDL-TGFA (ρ_{06}), calculated using models A or C, was consistently higher on the high-carbohydrate diet. These dietarily provoked changes, demonstrated in four paired studies in which the subjects served as their own controls, are probably the most reliable. In these cases, instead of dealing with the larger variability from patient to patient, we have two sets of data on a single system onto which a single perturbation has been imposed. Using model B, calculated net transport of VLDL-TGFA was smaller, increases were seen in only three or four studies on high carbohydrate diet and the increases were smaller than those calculated using the other models. As mentioned above, the actual physiological model very likely incorporates features of all three of the models used. That is to say, there may be a slower pathway through the hepatic biosynthetic compartment (No. 6) and some recycling from plasma back to the liver (No. 7), and a slower exchange of plasma FFA with extravascular FFA (No. 8). With such a combined model the calculated parameters, including ρ_{06} , would lie between the extremes calculated here using each separately. Consequently we feel that the weight of our evidence favors the conclusion that the high-carbohydrate diet does increase VLDL-TGFA production but the magnitude of the effect may well be less than that calculated using model A.

The mechanism by which VLDL-TGFA production might be increased on high-carbohydrate diets remains unclear. The present results suggest that plasma FFA are channeled to TGFA production at a higher rate without an increase in over-all FFA transport. Sailer, Sandhofer, Bolzano, and Braunsteiner (26) recently re-

ported studies with glucose- $U^{14}C$ showing that glucose carbon is *not* a significant source of fatty acid carbon in plasma triglycerides of type IV patients, and Havel (27) has shown that, certainly in normolipemic subjects, FFA are the primary or exclusive source of VLDL-TGFA. Thus, carbohydrate induction does not appear to depend on direct conversion of glucose to fatty acids, although increased supply of alpha-glycerophosphate may be relevant. One possibility, compatible with these results, is that on high-carbohydrate diet FFA taken up by the liver are "spared" from oxidation and thus shunted to triglyceride synthesis and thence channeled to VLDL-TGFA secretion.

In interpreting the changes in FFA metabolism it should be made clear that all irreversible FFA disappearance from compartment 1 other than that channeled to TGFA formation is encompassed in ρ_{01} . This includes FFA that are taken up by the liver but channeled into pathways other than into synthesis of VLDL-TGFA (e.g. oxidized to CO_2 or ketone bodies). Thus, although ρ_{01} was in every case increased on the high-carbohydrate diet, this does not necessarily imply a net increase in over-all FFA uptake by the liver. The total liver uptake might remain unchanged while a larger fraction of it was channeled to TGFA synthesis. Moreover, the rate of VLDL-TGFA production need not mirror the rate of triglyceride synthesis in the total liver. If it does (i.e., the triglycerides incorporated into the secreted VLDL come from a common pool of newly synthesized triglycerides) then the present result could be interpreted as being consonant with results in experimental animals demonstrating increased rates of triglyceride synthesis on high-carbohydrate diets (28).

Increments in VLDL-TGFA transport after carbohydrate have been noted previously (5, 9). In each of our carbohydrate induction studies it was observed that most of the increment in VLDL-TGFA transport occurred by way of the slow conversion pathway. It is understandable then that some investigators, evaluating triglyceride production rates only over a 1 hr interval might not detect any increase in glyceride production in response to high-carbohydrate diets (4).

The fractional catabolic rate of VLDL-TGFA (λ_{05}) was considerably lower in the hyperlipemic patients than in the normal subjects (Fig. 6). This was the most consistent kinetic difference between the two groups when comparison was made on the same diet. However, neither on the balanced diet nor on the high-carbohydrate diet was there any consistent difference between controls and type IV patients with regard to *net* VLDL-TGFA transport (Table VI and Fig. 7). For example, the type IV patient with the largest VLDL-TGFA pool on a balanced diet (12a in Table VI) showed a transport no different from that of a normal subject with a pool size

only one-fifth as large (76 $\mu Eq/min$ vs. 80 $\mu Eq/min$). On the high-carbohydrate diet, type IV patient No. 6 had a pool size five times that of normal control number 1, but their transport values were not distinguishable from one another. These findings are in accord with those of Sailer et al. (29) and of Havel (27). The important implication of this result is that our type IV patients are hyperlipemic not on the basis of an abnormally high rate of VLDL production but rather on the basis of a defect in rate of VLDL removal. The effect of a high-carbohydrate diet in these patients, as in normal subjects, is to increase VLDL production, but the increase does not appear to be any greater in Type IV patients than in normal controls. The difference seems to lie in the ability of the individual to cope with this increased production. We suggest that it is because of an as yet obscure defect in removal mechanisms that VLDL levels rise more strikingly in type IV disease during carbohydrate induction, as proposed previously by Knittle and Ahrens (30). However, we find nothing in the present results to suggest any basic difference in the responses of the lipoprotein producing system to carbohydrate feeding.

As mentioned above and discussed in the accompanying paper (12) $\lambda_{5,4}$ could be determined with only 30–50% precision. Variations in $\lambda_{5,4}$ could be almost completely compensated in fitting the data by joint but opposite variations in $\lambda_{3,1}$ and $\lambda_{0,5}$. For example a 20% increase in $\lambda_{5,4}$ could be compensated for by a 15% decrease in $\lambda_{3,1}$, $\rho_{3,1}$, and $\lambda_{0,5}$. If the hypothesis that $\lambda_{5,4}$ be invariant is relaxed, data indicating an apparent increase in $\lambda_{3,1}$ (and hence $\rho_{3,1}$) at a given $\lambda_{5,4}$ can be also fitted instead by an increase in $\lambda_{5,4}$ and a decrease in $\lambda_{0,5}$. Thus, the possibility that the high-carbohydrate diet simultaneously *increases* fractional rate of release of VLDL from the liver and *decreases* fractional rate of removal of VLDL from the plasma cannot be ruled out. However, an increase in glyceride productions from FFA ($\rho_{3,1}$) as a single prime perturbation seems more likely on physiological grounds.

Postheparin lipoprotein lipase activity in type IV disease is reported to be within normal limits (1). This test is relatively crude and may demonstrate deficits only in the more extreme cases, such as in type I hyperlipoproteinemia. If the normal results, however, are accepted as ruling out a deficit in the lipoprotein lipase-catalyzed mechanisms for lipoprotein removal, it must be postulated that there are additional and independent mechanisms regulating lipoprotein removal from the plasma.

Reaven, Hill, Gross, and Farquhar, on the other hand, have reported higher than normal rates of net transport of VLDL glyceride in subjects with elevated VLDL levels (5). In part, this may relate to the methods of kinetic analysis used, as previously suggested by Havel

(27) and discussed in the accompanying paper (12). In the individual with a normal fractional VLDL triglyceride removal rate ($\lambda_{0.5} \approx 0.017$) the turnover characteristics of the plasma VLDL pool are very similar to those of the immediate precursor pool (pool 4: $\lambda_{0.5} = 0.015$). The precursor pool in such a circumstance would be expected to influence the apparent decay rate of VLDL triglyceride activity more significantly, causing the slope to be less than it would be if the hepatic precursor pool turned over more rapidly than the VLDL triglyceride pool. This latter situation more closely characterizes the situation in the hyperlipemic subjects where the turnover of the precursor pool ($\lambda_{0.5} = 0.015$) is as much as four times that of the slowly turning over VLDL-TGFA ($\lambda_{0.5} = 0.0035$). In this situation the apparent initial slope of the specific activity curve following the peak would more nearly approximate the true fractional catabolic rate of VLDL-TGFA, and the estimated transports in these patients would be more accurate. Thus, there might appear to be a higher transport in the hyperlipemic subjects even though the actual transports are equal in the two groups. Alternatively, the difference in conclusion may relate to patient selection. In extending their studies, the Stanford group has found a number of subjects with very high VLDL triglyceride levels in whom net VLDL glyceride transport was no higher than that in mildly hyperlipemic subjects studied under the same conditions of high-carbohydrate diet (31).

Preliminary analyses demonstrated a typical precursor-product relationship between VLDL-TGFA and LDL-TGFA in the present studies, in agreement with results of Havel (11). Further exploration of this VLDL-LDL interconversion in patients with different lipoprotein patterns may yield insights into the underlying disturbances of the steady-state relationships.

The chylomicron TGFA specific activity data were significantly different from those for the VLDL-TGFA in the type I patients studied. The much slower decay of chylomicron radioactivity after the peak of the curve (Fig. 9) most likely reflects delivery of newly synthesized chylomicrons to the plasma from a precursor pool that turns over very slowly. However, it is possible that the fractional catabolic rates for chylomicrons in these patients are significantly less than those for VLDL-TGFA. In any case, it is obvious that in these individuals the chylomicron TGFA and VLDL-TGFA are kinetically distinct. Failure to remove chylomicrons during isolation of VLDL-TGFA could distort estimates of VLDL-TGFA transport and fractional catabolic rate.

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